

## METHOD FOR DETECTING TOTAL KETONE BODIES IN URINE

### PRIOR APPLICATIONS

This application is a divisional of application Ser. No. 08/429,292, filed Apr. 24, 1995, now U.S. Pat. No. 5,516,700 which is a continuation-in-part of application Ser. No. 08/068,956, filed May 28, 1993, (now abandoned).

### BACKGROUND OF THE INVENTION

This invention relates to a method and materials that are designed for use in automating urinalysis. This system is designed to analyze urine for its constituents by a method that is fully automated (does not require the use of manual methods such as refractometer, pH meter, dipsticks, or impregnated test strips). Automation as designed by this system is directed to the use of a self-operating instrument that is capable of handling multiple reagents designed for use on an automated analyzer system for the quantitative determination of total ketone bodies in urine.

It is known that the most common method for the analysis of urine is by the use of a manual technique known as a dipstick. This method for the analysis of urine is labor, time intensive, and costly among other detriments. The use of a dipstick for analysis of urine also relies on the subjective interpretation of the technician. The dipstick method requires the technician to submerge the dipstick in a sample of urine and remove it, wait a specified time, then compare the color development of the test on the dipstick to a color chart. Even more cumbersome methods involve the use of a refractometer, pH meter, or manual chemistry test.

The following list of assay devices utilizing prior art includes dry tablets, dipsticks, or impregnated test strips for the analysis of urinary constituents. None of the prior devices foresee or teach a multiple or single liquid reagent system designed specifically for auto-analyzers to analyze urinary constituents quantitatively.

One such U.S. Pat. No. 4,147,514 discloses test strips (dipsticks) for the detection of ketone bodies. The assay strips are made up of a chemical bonded to a cellulose pad on a strip. This is then dipped into a specimen sample. This method only determines ketone bodies qualitatively at its best, due to inability of the system to allow the use of standards and controls on the same strip to which the sample is applied.

Another such patent, U.S. Pat. No. 3,146,070 discloses analytical compositions in dry form on a bibulous carrier (dipstick) impregnated with a pH indicator for the determination of pH. This assay at best only determines pH qualitatively, due to the inability to use standards and controls located on the same strip for the same test sample to define and verify a quantitative determination.

Additionally, U.S. Pat. No. 4,318,709 discloses a device comprising a carrier matrix (dipstick) impregnated with the test means for specific gravity. This assay at best only determines specific gravity qualitatively, due to the inability to use standards and controls located on the same strip for the same test specimen. The prior art in this case also did not foresee the wide specimen to specimen matrix variations of real world urine samples including matrix components such as pH, and ionic strength, and the concomitant requirement of a multiple reagent system to effectively analyze urine for specific gravity in a liquid to liquid reaction. The normal pH value for urine can range from 4.5 to 8.0, which if using the prior dipstick method the results would be widely scattered

and inaccurate without a reagent component to neutralize this effect prior to completion of the assay.

Various devices are described in the literature for the determination of particular urinary constituents one by one with the use of carrier matrices (dipstick, microcapsule, filter paper, etc.). None of the prior art teaches or elucidates a means for determining by automated technology urinary constituents from a single sample of urine, via multiple tests that are reported simultaneously by an autoanalyzer using liquid reagents specifically designed for this family of instruments. As cited by the prior art, (in package insert literature) when evaluating laboratory test results, definitive diagnostic, or therapeutic decisions should not be based on any single result or method. However, the prior art states that dipsticks are affected by high specific gravity and substances that cause abnormal urine color such as phenyl ketone, or phthalein compounds and thus may affect the readability of the urinalysis reagent strips (dipsticks). In addition, sulfhydryl-containing compounds such as mercaptoethane sulfonate sodium, and levodopa metabolites may cause false positive results or atypical color reactions using the prior art.

### SUMMARY OF THE INVENTION

The automated urinalysis system of this invention offers a method for reducing the consumable materials and labor costs. The system also offers increased accuracy, sensitivity, and objective quantifiable determinations of urinary constituents for better diagnostic interpretation of the test results of urine, thus enabling a physician to provide better health care for the patient.

This invention satisfied many of the problems unanswered by the prior art: quantitative results, non-subjective results, reproducible results, increased accuracy, precision, sensitivity, carrier free reagents, reagents designed for autoanalyzer use, reagents uniquely designed for each particular urine analyte assay overcoming matrix problems previously unanswered by prior art, a method allowing vast improvement of test completion time (hundreds to thousands per hour). The present invention presents a fully automatable walk-away urinalysis system applicable to any discrete autoanalyzer currently in use, and obviously represents a marked advancement in the art of urinalysis. The clear cut object of the present invention is to provide a more comprehensive method for determining urinary constituents of total ketone bodies, such method specifically yielding improved health care.

Thus, it is a primary objective of the present invention to provide techniques for determination of urinary constituents of total ketone bodies at low chemically significant levels.

An additional object of this invention is to make available an advanced method for analyzing a sample of urine for the quantitation of its constituents on an autoanalyzer. The advanced ability of the automated urinalysis system to offer a means for automated analysis on urine is a significant improvement in the art of urinalysis.

Additionally, the object of this invention is to provide a comprehensive method which is broadly adaptable to a wide variety of automated analyzers presently in use in the industry which will increase accuracy, sensitivity, precision, and speed. An autoanalyzer allows for precise quantitative results beyond the scope and abilities of the prior art. An autoanalyzer, used in conjunction with the automated urinalysis reagents described herein, provides a system that produces an objective quantitative result of an unknown urine sample obtained from a linear standard curve determined by analysis of standards run on the instrument, and